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PRINCIPAL INVESTIGATOR: Clifton C. Ling, Ph.D.

CONTRACTING ORGANIZATION: Sloan-Kettering Institute
for Cancer Research
New York, New York 10021

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13. ABSTRACT (Maximum 200 Words) <p>The first year research has focussed on two studies. One is to develop a method that can non-invasively detect the hypoxic cell foci in malignant tumors. The iodo-azomycin galactoside (IAZG) was labeled with long-lived positron emitter, ¹²⁵I, as a PET radiotracer. In vitro experiments showed that the cellular uptake of IAZG was inversely related to the intracellular oxygen concentration. The pO₂-dependent IAZG uptake was investigated for 8 human cell lines including two breast cancer lines. This showed the highest uptake in two breast cancer cell lines. We are now evaluating the use of this agent in vivo together with regional blood flow in the tumor.</p> <p>Another study is to investigate the hypoxic cell fraction (HCF) as a function of tumor size. The well developed paired survival assay and the tissue pO₂ measurements (using an OxyLite system) were compared. The HCF was ~10% in small tumors (<200 mm³), but increased to ~35-50% in a tumor of 200 mm³. Above this tumor size, the HCF was consistent. As compared to paired survival assay, the direct pO₂ measurements tend to show the larger HCF, specifically in large tumors.</p>					
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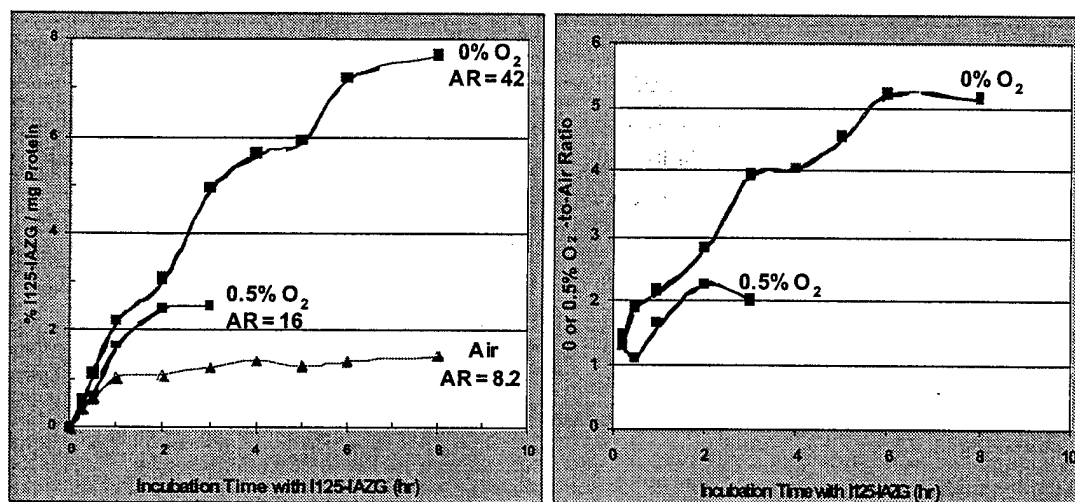
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The existence of hypoxia in human cancers, including breast cancer, is now unequivocal and hypoxic cells are substantially more radioresistant than normoxic cells. Failure to control localized cancer likely results from radioresistance of such hypoxic tumor clonogens to maximal dose levels tolerated by adjacent normal tissues and achievable with conventional radiotherapy. Tumor hypoxia also appears to be an important independent determinant of metastatic potential and therefore of relapse-free survival and overall clinical outcome in breast and other human cancers. It is therefore critical for improving curability of breast cancer to identify and localize hypoxic, radioresistant cells so that they may be specifically targeted with additional radiation or other therapies and so that the overall management of breast cancer patients may otherwise be appropriately modified. In the first year of this project, we have made two major studies.

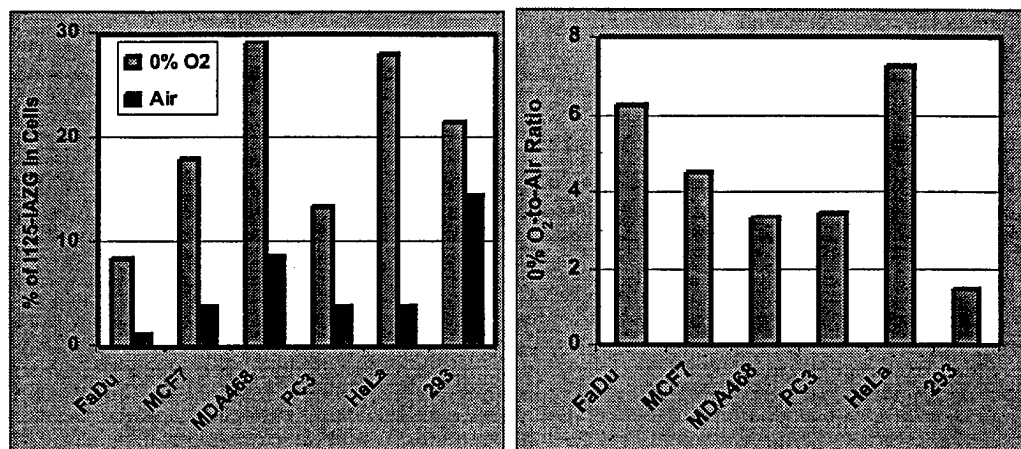
In collaboration with Drs. Donald Chapman and Richard Schneider of the Fox Chase Cancer Center, pre-clinical studies (*in vitro* and *in vivo* in tumor-bearing mice) have been performed with ^{125}I -labeled iodo-azomycin galactoside (IAZG). This can be a potential convenient surrogate for developing and characterizing IAZG labeled with the long-lived positron emitter ^{124}I ($T_{1/2} = 4.2$ d) as a PET radiotracer for non-invasively detecting, measuring, and determining the spatial distribution of tumor hypoxia in patients. As with misonidazole and other 2-nitroimidazoles, the cellular uptake of IAZG is inversely related to the intracellular oxygen concentration and closely parallels the pO_2 -dependent radiosensitivity of cells. Using FaDu squamous cell carcinoma cells (1 million cells at 37°C), the time-dependent cellular uptake was measured as a function of oxygen concentration (0%, 0.5%, and air (~25%)). As shown in the graphs below, maximal cellular uptake was achieved by 1, 2 and 6 hr in air, 0.5% O_2 , and 0% O_2 , respectively, with ~2- and 5-fold higher uptake in 0.5% and 0% O_2 , respectively, than in air. (Right-hand panel); the accumulation ratio (AR) is the μCi per gram of cells / the μCi per ml of medium. The rather prolonged time



course of accumulation of IAZG in hypoxic cells (*ie* 6 hr to achieve maximal uptake *in vitro*) suggests that better images should be obtained with long-lived ^{124}I -IAZG than, for example, with short-lived ^{18}F -fluoromisonidazole, where the 110-min half-life of ^{18}F limits useful imaging to only 2-4 hr post-injection.

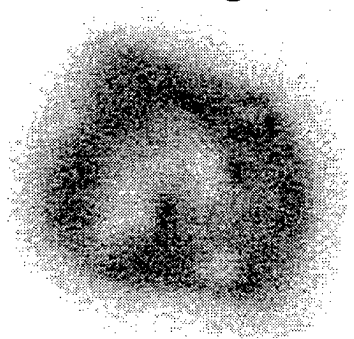
The pO_2 -dependent uptake (*ie* the maximal, or 8-hr, uptake) has been evaluated for a variety of human cell lines, including the MCF7 and the MDA468 breast cancer cell lines. As indicated in the graphs below, the accumulation ratios (ARs) of IAZG in hypoxic cells ranged from 8 to 50, with 2- to 8-fold high activity concentrations in hypoxic than in normoxic cells. Note that the uptakes (*ie* ARs) of IAZG at 0% O_2 and the differences in uptake between cells at 0% O_2 and in air for the two breast cancer cells, MCF7 and

MDA468, were among the highest of the six human tumor cell lines tested. This suggests that hypoxia in breast cancer may be imaged particularly well.



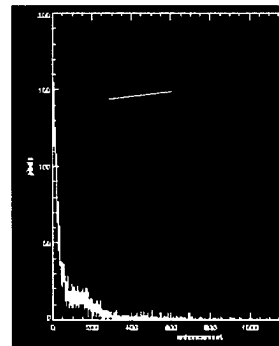
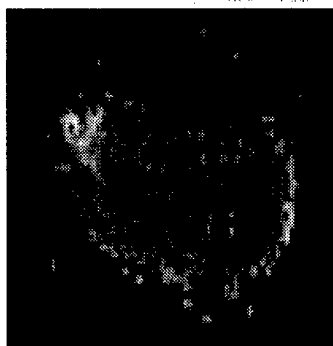
As with any systemically administered radiotracer, tumor uptake of IAZG *in vivo* may be limited by delivery, that is, by blood flow and thus may not be directly related to hypoxia. Therefore, in our development of radiolabeled IAZG as a tumor hypoxia-imaging agent, regional tumor blood is also being evaluated by a variety of methods. In the figure below, for example, the distribution of ¹²⁵I-IAZG in a FaDu tumor xenograft at 2 hr post-injection is shown in a phosphor-plate autoradiogram (left-hand panel). Immediately prior to tail-vein injection of the IAZG, a dynamic 4.7-T NMR study of regional tumor blood flow

¹²⁵I-IAZG Autoradiogram



~2 hr pi

Gd-DTPA Dynamic MRI



was performed following intravenous injection of Gd-DTPA. The terminal slope of the uptake portion of the signal-versus-time curve (right-hand panel), a measure of blood flow, was determined on a pixel-by-pixel basis and used to generate a parametric image related to regional tumor blood flow (middle panel). Note that the brightest portions of this parametric image, corresponding to the greatest blood flow, are restricted to the periphery of the tumor - precisely the areas of highest IAZG uptake within the tumor (indicated by the dark areas in the autoradiogram). Such assessment of regional tumor blood flow should allow us to normalize the uptake of IAZG for blood flow and thus "correct" the distribution of IAZG for the effect of tracer delivery. In this way, the equilibrium distribution of IAZG should more accurately reflect the distribution of hypoxic cells.

Another study in the first year is to investigate the hypoxic cell fraction (HCF) in tumors. After the transplantation of a half million tumor cells, this was investigated in various-sized tumors using two different methods. The first is the well-established paired survival method consisting of I) giving a large single radiation dose to air breathing mice and to mice sacrificed 10 minute prior to irradiation (to induce hypoxia), II) excising the tumor and dispersing into single cell suspension, and, III) plating for colony formation assay. The hypoxic fraction is given by the ratio of plating efficiencies of the tumors from the air-breathing animals and from the sacrificed animals, respectively. The second method is to directly measure the tissue pO_2 in tumors. We have been using the OxyLite Model 4000 system consisting of 4 oxygen sensors (Oxford Optronix, Oxford, UK). The sensors (probes, BF/O/2.5/NS) rely on the measurement of the oxygen-quenched lifetime of a luminescent molecule (luminophor) mounted at the tip of an optical fiber (outside diameter is 0.230 mm). Similar studies are in progress using the human FaDu tumor.

Results obtained in the FSa-II tumors are presented in the figure 1. The paired survival method showed that the HCF in a small tumor such as 100 mm^3 was less than 10%, but increased rapidly to 35-50% with tumor growth up to 200 mm^3 and remained constant up to the tumor size of ~1,500 mm^3 at which the experiment was terminated. As compared to these obtained by the paired survival method, the values obtained by the tissue oxygen measurements were greater in the tumor larger than 500 mm^3 , although those in the tumor size ranging from 100 to 500 mm^3 was very similar.

A table shown below showed the tissue pO_2 of five FSa-II and four FaDu tumors with an average diameter of 8-10 mm. As in the aforementioned study, a probe was inserted into a tumor through a 25-gauge needle and the tissue pO_2 was measured 1 mm stepwise. The first reading was made at 1 mm depth from the tumor tissue surface and continued until the tip reached the other end of the tumor. At least 35 measurements were made in a tumor. The percentages of readings (frequency) at various pO_2 values are tabulated below. In this range of tumor size, the FaDu tumors contain more hypoxic cells than the FSa-II tumors. This is also shown in Figure 2 for the FSa-II tumors in 5 mmHg step-wise.

Figure 1. Hypoxic cell fractions measured by two Different methods as indicated in the figure.

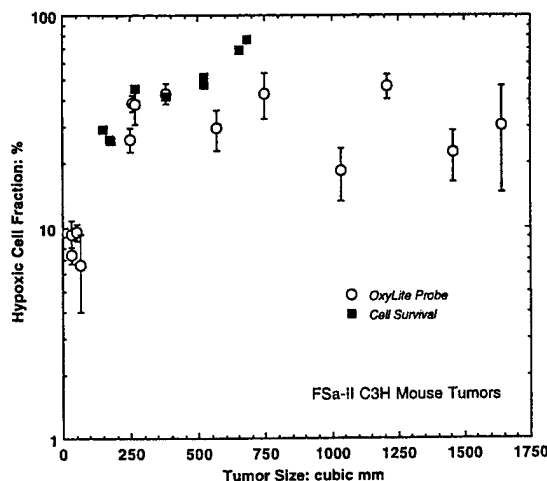
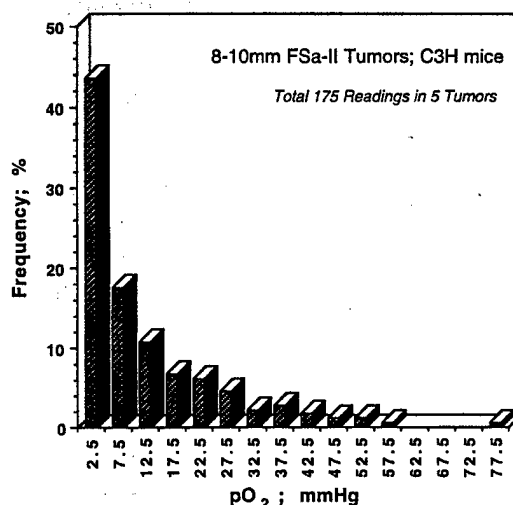


Figure 2. % pO_2 readings in 5 mmHg stepwise.



pO_2 (mmHg)	< 2.5	2.6-5.0	5.1-10.1	10.1-20.0	> 20.1
FSa-II tumor	42.9%	5.7%	12.5%	18.3%	20.5%
FaDu tumor	53.9%	10.5%	9.9%	15.5%	10.2%